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SHORT COMMUNICATION

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Selection on worker honeybee responses to queen pheromone (*Apis mellifera* L.)

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Abstract Disruptive selection for responsiveness to queen mandibular gland pheromone (QMP) in the retinue bioassay resulted in the production of high and low QMP responding strains of honeybees (*Apis mellifera* L.). Strains differed significantly in their retinue response to QMP after one generation of selection. By the third generation the high strain was on average at least nine times more responsive than the low strain. The strains showed seasonal phenotypic plasticity such that both strains were more responsive to the pheromone in the spring than in the fall. Directional selection for low seasonal variation indicated that phenotypic plasticity was an additional genetic component to retinue response to QMP. Selection for high and low retinue responsiveness to QMP was not an artifact of the synthetic blend because both strains were equally responsive or non-responsive to whole mandibular gland extracts compared with QMP. The use of these strains clearly pointed to an extra-mandibular source of retinue pheromones (Pankiw et al. 1995; Slessor et al. 1998; Keeling et al. 1999).

Introduction

Pheromones are a principal form of communication and social cohesion used among social insects. They are used to communicate or organize a broad spectrum of functions including alarm, defense, recruitment, recognition of nestmates, caste, sex, age, and to regulate reproduction (Vander Meer et al. 1998). The last 30 years of pheromone research has revealed that social insects have numerous exocrine glands producing an astonishing number of chemicals functioning as multi-component blends operating on multiple levels as releasers of behavior and/or as physiological primers that alter endocrine and/or reproductive systems (Hölldobler and Wilson 1990). The core of chemical ecology is the bioassay coupled with chemical analysis resulting in the isolation of chemical complexes involved in releasing or priming biological functions.

Worker honeybees vary in their response to queen mandibular pheromone (Pankiw et al. 1994). Retinue behavior is the releaser response to QMP characterized by frequent antennating, licking, grooming and feeding activities directed toward the queen or a glass bulb spotted with QMP (Kaminski et al. 1990). Queen pheromone is dispersed through the nest in part by the movement of the queen and through serial transmissions by retinue bees as worker-to-worker transmissions (Naumann et al. 1991, 1992). As a primer pheromone QMP suppresses the queen rearing activities of workers (Winston et al. 1991; Pettis et al. 1995), might inhibit worker ovary development (Willis et al. 1990; Lin et al. 1999), and delays worker foraging ontogeny (Pankiw et al. 1998).

Here we demonstrate the development of new bioassay tools through disruptive selection on QMP retinue response to produce high and low responding strains and concurrently we performed directional selection for low seasonal variation in retinue response to QMP. Finally, we demonstrate that the selected strains were high and low responding to whole extracts of queen mandibular glands and thus were not an artifact of the synthetic blend.

Materials and methods

Retinue response to QMP was quantified in a retinue bioassay described in detail by Pankiw et al. (1994). Each replicate of the

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bioassay consisted of 15 workers in a bioassay arena. The sum of workers contacting a glass lure spotted with 10 μl of 10^{-3} queen equivalents of QMP (Slessor et al. 1988) at 30-s intervals over a 5 min period quantified retinue response. An accompanying control bioassay quantified retinue response to a glass lure spotted with 10 Fl of solvent (methanol or 2-propanol). Unless otherwise mentioned, mean lure contacts represent ten replicates of the retinue bioassay per colony.

We conducted a disruptive selection program beginning with the selection of initial stocks. Initial stocks were derived from five different sources; (1) Babe's Honey, Victoria, B.C., Canada ($n=27$), (2) Aussie Apiaries ($n=25$) and (3) Barden Apiaries ($n=11$), New South Wales, Australia, (4) Whiteline Queens, New Zealand ($n=23$), and SFU stock ($n=31$). Colony-level responsiveness was based on the mean of five bioassay replications. Colonies within sources were ranked highest to lowest responding. The highest and lowest colonies from each source were assayed once more 2 months later immediately prior to rearing queens from the selected sources. Any colonies that did not display their initial phenotype were rejected from the selection and replaced with colonies that did. Ten high-responding colonies (two per source) and ten low-responding colonies (two per source) were selected. Each generation of virgin queens was raised using standard methods and each queen was instrumentally inseminated with semen from a single drone (Laidlaw and Page 1996). The Barden line was eliminated from the program after the first generation due to difficulties in maintaining fecund queens. Therefore, five maternal sub-lines were initiated but only four lines were maintained for three generations.

The following year initial crosses were performed creating high and low lines A–D and V–Z, respectively. The mating design used throughout the program was a half-sister closed circular mating design (Laidlaw and Page 1996). In this design a queen has a hermaphroditic parent and is one in turn. Queens are hermaphroditic because males are haploid derived from unfertilized eggs and therefore represent a queen's gametes. Within a generation all progeny of each queen are half sisters to the progeny of each other queen. When queens were approximately 10 days old they were each instrumentally inseminated with semen from a single drone. Colonies were allowed to grow and were managed in the usual manner to control diseases, parasites and swarming. Eight colonies per line per strain were assayed (total of 64 colonies). Colony-level responsiveness was based on five replicates of the QMP retinue response bioassay. Selected colonies were reassayed (ten replicates per colony) prior to queen rearing the following spring. Colonies that did not display a previously assigned high or low phenotype were not used as queen sources.

The second generation was derived from virgin queens reared from the queens heading the highest and lowest responding colonies per line above. Queen rearing and instrumental inseminations were conducted as above. The number of colonies assayed from each cross is indicated in parentheses. High strain crosses were: A♀×B♂ (5), B♀×C♂ (3), C♀×D♂ (4), D♀×A♂ (7). Low strain: W♀×Z♂ (4), V♀×W♂ (3), Y♀×V♂ (7), Z♀×Y♂ (2). The following high and low line crosses were made to produce colonies with generation 3 workers. High strain crosses were: A♀×C♂ (7), B♀×D♂ (2), C♀×A♂ (5), D♀×B♂ (8). Low strain crosses were: W♀×V♂ (5), V♀×Y♂ (3), Y♀×Z♂ (6), Z♀×W♂ (2). The entire breeding program from initial selections to the production of third generation workers required 4 years because the short spring and summer seasons in Canada allowed only one mating per year.

Those colonies displaying the least seasonal variation were used to parent the subsequent generations. The two highest and two lowest responding colonies per strain, line, and generation as determined by the spring bioassays (June to early July) were assayed once more in the fall (late August to mid-September). Consequently we applied two forms of selection pressure concurrently to produce the high and low strains.

Workers from ten high and ten low strain colonies of the second generation were tested for response to the whole gland extract, synthetic QMP, and control (methanol) to determine whether the selection was based on an artifact of the synthetic blend or to retinue response to queen mandibular pheromone. Whole gland extracts were prepared from excised glands of three mature laying queens. The glands were macerated and extracted twice in HPLC-grade methanol to give a combined total extract of 100 μl per queen. A portion of this extract was diluted to yield a 10^{-3} queen equivalents in 10 μl.

Results

The 117 colonies of the initial stocks had an average retinue response of 12.6±0.7 (SE) and responses were normally distributed (Sokal and Rohlf 1995). Queens selected to parent the first generation of high and low strain lines had colony phenotypic values ±1 SD from the mean, respectively. This comprised 18% low responding (mean response ≤5.0) and 17% high responding (mean response ≥23) of the initial population.

QMP retinue responses between strains, within generations, were significantly different; initial selection, high 20.5±2.7 (SE), low 6.3±0.9; generation 1, high 18.5±1.1, low 6.6±0.9; generation 2, high 34.2±2.9, low 3.7±0.6; generation 3, high 38.6±2.1, low 4.2±0.5, Mann-Whitney U, $P<0.05$ (Fig. 1). Spearman rank correlation analyses on retinue responses by generation showed that the amount of variation explained by genotype increased with each generation: initial selection $\rho=0.43$, $P<0.0001$; generation 1, $\rho=0.49$, $P<0.0001$; generation 2, $\rho=0.69$, $P<0.0001$; generation 3, $\rho=0.72$, $P<0.0001$ (Sokal and Rohlf 1995). Two generations of selection were necessary to eliminate significant spring and fall differences in the retinue responses of the high strain (Fig. 2), Mann-Whitney $U=4.0$, $P>0.1$, while for the low strain seasonal differences were eliminated in the first generation, Mann-Whitney $U=3.0$, $P>0.05$ (Fig. 2) (Sokal and Rohlf 1995).

Within-strain responses to synthetic QMP and gland extract were not significantly different (Mann-Whitney

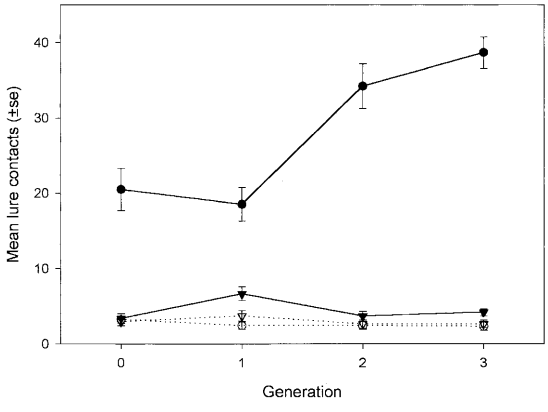

Fig. 1 Retinue responses to queen mandibular pheromone (QMP) by initial crosses and three generations of the high and low strains. High strain responses to QMP are indicated with *closed circles* and solvent control responses with *open circles*. Low strain QMP responses are indicated with *closed triangles* and solvent control responses with *open triangles*

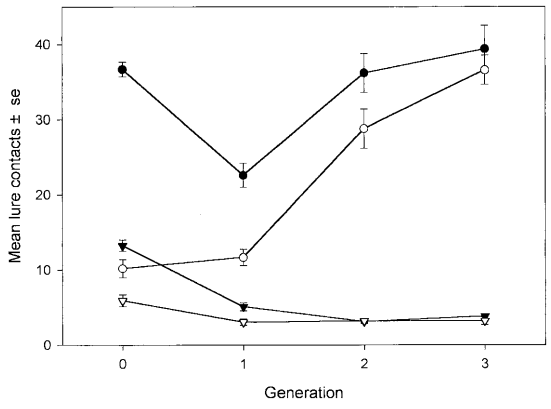

Fig. 2 Selection response to reduced seasonal phenotypic plasticity in retinue response to queen mandibular pheromone in the high and low strains. High strain spring responses are indicated with *closed circles* and fall responses with *open circles*. Low strain spring responses are indicated with *closed triangles* and spring responses with *open triangles*

U test, $P>0.05$) (Sokal and Rohlf 1995). High strain mean retinue response to QMP was 25.1 ± 2.6 (SE), to the gland extract 20.3 ± 2.2, and the solvent control 2.5 ± 0.3. Low-strain mean retinue response to QMP was 4.7 ± 1.1, to the gland extract 4.5 ± 1.3, and the solvent control 2.3 ± 0.2. High and low strain responses were significantly different from each other in their responses to both QMP and whole gland extract ($P<0.05$). Additionally, low strain responses to the pheromones and the solvent were not significantly different ($P>0.05$) whereas high strain responses were significantly different ($P<0.05$).

Discussion

For the first time, high and low pheromone responding bees have been selected and used as tools in pheromone ecology. The development of these strains and their use highlighted some important aspects of retinue response that might have been overlooked or difficult to discover. The selection for extreme phenotypes not only demonstrated a genetic component to pheromone response, but that there was an underlying seasonal plasticity to retinue response that also had a genetic component. High and low QMP responding strains were equally responsive to a queen in a colony (Pankiw et al. 1995). This suggested that low strain bees were attracted to extra-mandibular gland compounds emanating from the queen. Low QMP responding phenotypes were used as a pheromone detection tool (Slessor et al. 1998; Keeling et al. 1999) where unselected "wild-type" bees would have been of little or no value.

The initial population of colonies tested expressed a wide range of variability in retinue responses to QMP (Fig. 2). There was a rapid response to disruptive selection demonstrating that there is a strong genetic component to retinue response to QMP in honeybees. Retinue responses in the first generation of high and low strains showed phenotypes that were not as high or low responding as their parents. This is a common phenomenon observed in selection programs (Harlt 1988). The means of the first generation are not as phenotypically desirable for two reasons: (1) some of the selected colonies do not have favorable genotypes and the desirable phenotype demonstrated was a result of chance, and (2) alleles, not genotypes are transmitted to the offspring. Favorable genotypes were disrupted by segregation and recombination (Harlt 1988). There was some inbreeding in the third generation but we do not believe this affected retinue response. High and low strain responses are not affected by rearing environment (Pankiw et al. 1994). Response is modulated by the amount of pheromone presented in the bioassay but constrained by genotype such that high strain bees are more responsive than low strain bees at all doses (Pankiw et al. 1994). Additionally, there is no relationship between queen pheromone production and worker response (Pankiw et al. 1994).

The seasonal variation in retinue responses in both strains can be viewed as seasonal phenotypic plasticity. We used directional selection because our goal was to produce phenotypically stable strains. However, disruptive selection for seasonal plasticity to create tools to examine the role of pheromone response plasticity would have been equally interesting (Schlichting and Pigliucci 1998), and potentially a fruitful area to study. Both strains were most responsive to QMP in the spring, suggesting a reduced response threshold to QMP at that time. Spring is a time of year when colonies grow and make the decision to swarm (reproduce) or not (Winston 1987). Small swarms or swarms that issue late in the season have low survival probabilities (Lee and Winston 1985). Seasonally related retinue response thresholds may be associated with queen-rearing inhibition and colony reproduction.

Changes in retinue response to QMP occur over evolutionary and individual time scales. Changes occurred in a time scale of generations, an evolutionary process. Changes associated with seasonal environment as a developmental stimulus turns genetic variability into phenotypic variability, an additional evolutionary process (Slessor et al. 1988; Pankiw et al. 1994). Response to the queen and QMP changes with age, a developmental process (Seeley 1979; Pham-Delègue et al. 1991). Responses also change with the amount of pheromone, an immediate response (Pankiw et al. 1994). Thus, modulation of retinue response to QMP occurs in different times scales, indicating different mechanisms for modulating a common sensory-physiological pathway. The modulation mechanisms, sensory-physiological pathway, and how these factors and extra-mandibular pheromones enable social regulation, have yet to be identified.

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